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Long-term effects of high intensity resistance and endurance exercise on plasma leptin and ghrelin in overweight individuals: the RESOLVE Study

Angelo Tremblay^{1,2}, Frédéric Dutheil^{3,4}, Vicky Drapeau^{1,5}, Lore Metz^{6,7}, Bruno Lesourd⁸, Robert Chapier⁹, Bruno Pereira¹⁰, Julien Verney^{6,7}, Julien S Baker¹¹, Agnes Vinet¹², Guillaume Walther¹², Philippe Obert¹², Daniel Courteix^{6,7}, David Thivel^{6,7}

¹*Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec City, Canada*

²*Department of Kinesiology, Université Laval, Quebec City, Canada*

³*CNRS, LaPSCo, Physiological and Psychosocial Stress, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Preventive and Occupational Medicine, WittyFit, Université Clermont Auvergne, Clermont-Ferrand, France.*

⁴*Faculty of Health, School of Exercise Science, Australian Catholic University, Melbourne, VIC, Australia.*

⁵*Department of Physical Education, Université Laval, Quebec City, Canada*

⁶*Clermont Auvergne University, EA 3533, Laboratory of the Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P), Clermont-Ferrand, France.*

⁷*CRNH-Auvergne, Clermont-Ferrand, France.*

⁸*CHU G. Montpied, F-63000 Clermont-Ferrand, France*

⁹*Thermalia Center, F-63140 Châtelguyon, France*

¹⁰*Clermont-Ferrand University hospital, Biostatistics unit (DRCI), Clermont-Ferrand, France*

¹¹*Institute of Clinical Exercise and Health Sciences, School of Science and Sport, University of the West of Scotland, Hamilton, Lanarkshire, Scotland, United Kingdom*

¹²*Avignon University LAPEC EA4278, F-84000 Avignon, France*

Running head: Hormonal response to exercise

Corresponding author

Angelo Tremblay, Ph.D.

Department of Kinesiology, PEPS

2300, rue de la Terrasse, Université Laval

Québec, Canada, G1V 0A6

Phone: 418-656-7294

E-mail: angelo.tremblay@kin.ulaval.ca

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Authors' implication: all authors significantly took part in the study from its conception to the analyses of the data and writing of this paper.

38 **Abstract**

39 **Objective:** To evaluate the effects of high intensity resistance and endurance exercise on body
40 composition and plasma leptin and ghrelin concentrations in overweight individuals.

41 **Methods:** 100 participants were randomly assigned to three exercise interventions: high resistance-
42 low aerobic (Re), low resistance-high aerobic (rE), and low resistance-low aerobic (re). Interventions
43 began with 3 weeks of residential supervision (Phase1) after which participants had to manage the
44 physical activity programs individually (Phase2). Body composition and plasma variables were
45 measured at baseline and after Phase 1 as well as after 3, 6, and 12 months.

46 **Results:** Significant decreases in weight and body fat were observed after Phase 1 ($p<0.001$), and
47 continued at a lower rate for up to 3 months, and then remained stable for the rest of the protocol.
48 Once a body weight plateau was reached, body fat loss after the Re and rE conditions exceeded by
49 1.5-2 kg the fat loss observed in the re condition ($p<0.05$). Leptin was significantly decreased after
50 Day 21 and Month 3 ($p<0.001$) and remained stable for the rest of the study. Ghrelin was significantly
51 increased after Day 21 and Month 3 ($p<0.001$) and returned to a level comparable to baseline between
52 Month 6 and 12 when body weight and fat had reached a plateau.

53 **Conclusions:** This study reinforces the idea that an increase in exercise intensity may accentuate
54 body fat loss before the occurrence of a body weight plateau. Resistance to further fat loss was
55 accompanied by a decrease in plasma leptin and an increase in plasma ghrelin.

56

57 **Key words:** obesity, physical activity, energy, appetite, hormones, fat

58

59 Introduction

60 Physical activity has been traditionally studied in the etiology and management of obesity because of
61 its potential to increase energy expenditure. Specifically, research has aimed to determine if there is
62 a deficit in exercise-induced thermogenesis in obese individuals whereas numerous clinical trials have
63 tested physical activity as a calorie-burning agent in weight loss interventions. However, we have
64 also shown that calorie for calorie, high intensity exercise is more susceptible to induce a negative
65 energy balance than a low to moderate intensity physical activity (27, 29). This effect seems to be
66 explained by post exercise adaptations such as an increase in resting metabolic rate (32) and an
67 incomplete compensation in energy intake (14). From a mechanistic standpoint, an increase in beta-
68 adrenergic stimulation was found to be involved in these post exercise effects (25, 32). Furthermore,
69 the discovery of hormonal messengers such as leptin and ghrelin has enriched the study of
70 mechanisms that may underlie the impact of exercise training on energy balance.

71 Following its discovery in 1994 (34), leptin was shown to promote a negative energy balance via
72 anorectic and thermogenic effects (11, 18). Leptin was also found to be reduced in exercise-trained
73 individuals (12) as well as following exercise protocols (13). This is concordant with the study of
74 Pasman et al. (22) who reported a significant association between the number of hours of exercise
75 and plasma leptin following a 16-month protocol combining diet and exercise training (22). Leptin
76 has been found to increase with aging, altogether with increased leptin resistance (Rigamonti et al.,
77 2002). In a recent meta-analysis, Rostas and collaborators found that exercise training favors
78 decreased leptin concentrations in middle aged and older overweight and obese individuals, resistance
79 training inducing a more pronounced leptin reduction than aerobic training alone. This suggests a role
80 for exercise modality on plasma leptin which may be attributable to the different stimulus provided
81 by resistance and aerobic exercise (Rostas et al., 2017).

Commented [D1]: Rigamonti AE, Pinelli AL, Corrà B, et al. Plasma ghrelin concentrations in elderly subjects: comparison with anorectic and obese patients. J Endocrinol. 2002;175:R1-R5.

Commented [D2]: PLoS One. 2017 Aug 15;12(8):e0182801. doi: 10.1371/journal.pone.0182801. eCollection 2017. In middle-aged and old obese patients, training intervention reduces leptin level: A meta-analysis. Rostas I¹, Póti I^{1,2}, Mátrai P^{1,2}, Hevvi P^{1,3,4}, Tenk J¹, Garami A¹, Illés A¹, Solymár M¹, Pétervári E¹, Szűcs A⁵, Párniczky A⁶, Pécsi D¹, Rumbus Z¹, Zsiborás C¹, Füredi N¹, Balaskó M¹.

82 The study of variations in plasma ghrelin is also worth consideration to understand the effects of
 83 exercise training in obese individuals. Ghrelin is an orexigenic hormonal messenger that increases in
 84 blood before eating and immediately decreases after food consumption (6). Accordingly, Cummings
 85 et al. (7) reported an increase in plasma ghrelin in obese individuals subjected to diet-induced weight
 86 loss. In response to exercise training, ghrelin was found to be increased when the intensity of the
 87 exercise stimulus was low to moderate (24). On the other hand, some evidence indicates that high-
 88 intensity exercise can reduce plasma ghrelin (2, 30). With age, ghrelin concentration has been found
 89 to decrease as well as the ghrelin signaling pathways (Rigamonti et al., 2002). Markofski et al. found
 90 that a 12-week aerobic + resistance training was able to increase fasting ghrelin concentrations by
 91 47% in 70 years old individuals (Markofski et al., 2014). Interestingly recent results suggest that the
 92 effect of exercise training on ghrelin concentration might depend on the volume of exercise, with 4
 93 months of moderate dose of aerobic exercise favoring reduced ghrelin while it remained unchanged
 94 in response to a low dose training program in old women (Bowyer et al., 2018).

95 Certain studies have examined the impact of exercise on plasma ghrelin in a context where the
 96 opposite effect of body weight loss was expected to be significant. For instance, Kim et al. (15)
 97 observed that body weight and percent body fat decreased in obese children after a 12-week aerobic
 98 and resistance exercise training while total ghrelin increased by 30.4% and acyl ghrelin did not
 99 change. Martins et al. (19) found that body weight decreased while plasma acyl ghrelin and appetite
 100 increased after 12 weeks of exercise training in sedentary obese women. This is concordant with
 101 results reported by Santosa et al. (23) and Zahorska Markiewicz et al. (33).

102 Taken together, these observations show that both leptin and ghrelin contribute to the metabolic
 103 regulation underlying the effects of exercise training. In addition, available literature reveals that this
 104 regulation can be modified by time, modalities of exercise practice and variations in body fat. From

Commented [D3]: Exercise training modifies ghrelin and
 adiponectin concentrations and is related to inflammation in
 older adults
 Markofski MM, Carrillo AE, Timmerman KL, Jennings K, Coen
 PM, Pence BD, Flynn MG.
 J Gerontol A Biol Sci Med Sci. 2014 Jun;69(6):675-81.

Commented [D4]: J Behav Med. 2018 Nov 17. doi:
 10.1007/s10865-018-9990-z. [Epub ahead of print]
 The influence of exercise training dose on fasting acylated
 ghrelin concentration in older women.
 Bowyer KP^{1,2}, Carson JA¹, Davis JM¹, Wang X³

105 a clinical standpoint, this observation has significant potential implications for obesity management
106 that deserve further investigations. In the present study, we report relevant data collected in the
107 RESOLVE Study (9) to document the impact of high-intensity resistance and endurance training
108 combined with dietary guidelines on plasma leptin and ghrelin in overweight individuals tested at
109 different time intervals over a 12-month intervention.

110 **Methods**

111 *Subjects*

112 A sample of 100 individuals (44 men, 56 women) were recruited to participate in this study via
113 advertisement. As previously described (9), the following inclusion criteria had to be respected to
114 permit eligibility: aged between 50 and 70 years, having a diagnosis of metabolic syndrome (METs)
115 (1), being overweight and sedentary, having maintained a stable body weight and medical treatment
116 over the last 6 months, to be post-menopausal for women, not to have restricted diet over the previous
117 year and to have completed a satisfactory VO₂max test. Additionally, the participants had to be
118 exempt from some diseases having the potential to interfere with the metabolic outcome of this study
119 (9). All subjects gave their written consent to participate in the protocol.

120 *Design*

121 This study is part of the larger RESOLVE project that is a clinical trial designed to investigate the
122 effects of a lifestyle intervention combining exercise and nutritional diet in individuals with metabolic
123 syndrome. The full experimental design, population recruitment procedure, eligibility criteria,
124 measurements as well as compliance and drop-out rates have been previously reported (5, 9, 31).
125 Briefly, all participants underwent a comprehensive medical screening procedure to ensure their
126 ability to complete the entire protocol. Eligible subjects were free from clinical signs of heart failure,
127 coronary artery disease, previous cerebrovascular events, atrial fibrillation and congenital heart

128 disease and were not using medication altering body weight or had not been on any restrictive diets
129 in the previous year. The participants were randomly assigned to one of the three exercise
130 interventions differing from each other by the relative intensity of resistance (R) and endurance (E)
131 sessions (with stratification according to age, sex and body mass index), for 3 weeks: i) Condition Re
132 was a high resistance-moderate endurance exercise whose modalities imposed 10 repetitions at 70%
133 of 1 maximal repetition and 30% VO_2 peak for endurance exercise; ii) Condition rE was performed
134 at moderate resistance (30%) and high endurance (70%) intensity; iii) Condition re was the reference
135 condition with both resistance and endurance exercise being performed at 30% maximal reference
136 values. It is important to note that evaluators were blinded relative to the condition being assigned to
137 each subject. For the following 12 months, the participants were all requested to maintain the same
138 training program individually while relying on guidelines and exercise prescription that they had
139 received in Phase 1.

140 Anthropometric measurements, body composition (DXA), blood samples, clinical and physical
141 assessments, daily food intake (3-day food diary) and various health-related questionnaires, were
142 performed at baseline (D0), after the 3-week intervention (D21), 3, 6 and 12 months after (M3, M6
143 and M12). The study was approved by the human ethics committee from the University Hospital of
144 Saint-Etienne, France. The intervention was registered with the American National Institutes of
145 Health database: No. NCT00917917.

146 ***Measurements***

147 ***Anthropometric measurements and body composition***

148 The participants weight and height was recorded while wearing light clothes and standing bare-
149 footed, using a digital scale and a standard wall-mounted stadiometer respectively. BMI was
150 calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured at

151 midpoint between sub-costal and supra-iliac landmarks (21). Fat mass (FM) and fat-free mass (FFM)
152 were assessed by dual-energy X-ray absorptiometry (DXA) following standardized procedures
153 (QDR4500A scanner, Hologic, Waltham, MA, USA).

154 ***Daily energy intake.***

155 Participants were asked to complete a 3-day dietary recall that was explained and detailed to them by
156 a member of the investigation team (including 2 week-days and 1 weekend day). The participants
157 were asked to indicate as precisely as possible all the details regarding the food ingested at each meal
158 and in-between meals. During their first visit, a specialized dietitian detailed the diary and the
159 methodology used to fill it in to the participants and the diaries were reviewed afterward with the
160 participants and the dietitian during a 45 minutes interview. The records have been analyzed by a
161 trained dietitian using the NutriLog software (Nutrilog SAS, Paris, France).

162

163 ***Blood samples***

164 Fasting blood samples were drawn between 7.00 and 7.30 a.m. by an experienced nurse, aliquoted
165 and stored at -80°C until analysis. Basic biological assays were performed in the biochemistry
166 laboratory of the University Hospital of Clermont-Ferrand, France. Total ghrelin and leptin were
167 assayed by ELISA using commercial kits (Millipore, Billerica, MA, USA). Sensitivity, intra- and
168 interassay coefficients of variation were respectively 30 pg/ml, 1.1% and 6.9% for total ghrelin and
169 0.16 ng/ml, 5.1% and 7.4% for leptin.

170 ***Detailed lifestyle intervention***

171 As previously described by Dutheil et al. (9), the protocol for each condition was divided in two
172 phases:

173 Phase 1: This phase elapsed over 3 weeks during which participants stayed in a residential
174 establishment where their exercise program and food intake were supervised. In each condition,
175 participants had to perform 15-20 hours of exercise per week that included 90 minutes of daily aerobic
176 exercise plus four 90 minute weekly resistance exercise sessions. As indicated above, the conditions
177 differed by the relative intensity of either resistance or endurance exercise. A Polar S810 system was
178 used to record and store heart rate values. Endurance training included aquagym, cycling and walking
179 whereas resistance training was based on 8 exercises with free weights and traditional muscular
180 development equipment. For each exercise, participants had to perform 3 series of 10 repetitions.
181 Maximal test were realized at baseline to determine the individual capacities of each participants.
182 Regarding the resistance intervention, tests were realized for each of the selected exercises in order
183 to determine the participants 10RM (maximal 10 repetitions). The training intensity increased from
184 65% to 85% of 10 maximal repetitions for Re, whereas rE and re remained at 30% of 10 maximal
185 repetitions. Resistance training was done 4 times a week and consisted of 15 min warm-up followed
186 by height exercises with free weights and traditional muscle building equipment. Exercises were high
187 pulley machine (lower back), seated row (upper back and trapezius), bench press (chest), chest fly
188 (chest), squat press (legs), leg extension machine (quadriceps), dumbbell curl (biceps brachial),
189 triceps pushdown on high pulley (triceps brachial). Each exercise was performed for three sets of 10
190 repetitions with 1 min rest interval A VO₂peak test was also realized by each participants at baseline.
191 Intensity of the endurance sessions increased gradually from 40 to 75% of VO₂max from week 1 to
192 week 3 for rE, whereas Re and re remained at 30% of VO₂max. Throughout the residential program,
193 participants received both standard and personalized meals prescribed by dietitians. Protein intake
194 was set at 1.2 g/kg body weight/d and accounted for 15-20% daily energy intake. Lipid and
195 carbohydrate intake provided 30-35 and 45-55% daily energy intake, respectively. Total daily energy
196 intake was calculated to promote a 500 kcal daily negative energy balance.

197 Phase 2: This phase covered the remaining part of the one-year intervention, i.e. between Day 21
198 (D21) and the end of Month 12. During this period, participants were requested to maintain the same
199 training program individually while relying on guidelines and exercise prescription that they had
200 received in Phase 1. They were met by the exercise coach and the dietitian at months 3, 6, and 12
201 (M3, M6, M12). As previously described (9), a compliance score was determined on the basis of the
202 number of food questionnaires returned (score from 0 to 12 i.e. 12 = 100%) and the number of training
203 sessions undertaken per week (score from 0 to 4, i.e. 4 = 100%). The overall compliance score was
204 the mean of these two scores (nutrition and physical activity).

205 *Statistical analysis*

206 Statistical analyses were carried out using the statistical software Stata (version 13, StataCorp,
207 College Station, US). All statistical tests were conducted for a two-sided type I error at 0.05.
208 Continuous variables were described as mean and standard-deviation, according to statistical
209 distribution (assumption of normality studied using Shapiro-Wilk test). Repeated correlated data were
210 analyzed using random-effects models to study fixed effects group (Re, rE, re), time-point evaluation
211 (baseline, D21, M3, M6, M12) and their interactions taking into account between and within subject
212 variability (as random-effect). A Sidak's type I error correction was applied to take into account the
213 multiple comparisons. Where appropriate, the normality of residuals was studied using Shapiro-Wilk
214 test. If necessary, a logarithmic transformation was proposed to achieve the normality of dependent
215 outcome. Furthermore, to determine if the treatment effects on plasma leptin and ghrelin were
216 independent from variations in BMI, lean and fat mass, multivariable random-effects models were
217 performed with these variations as covariates. Concerning non-repeated data, the following statistical
218 tests were performed: Student t-test or Mann-Whitney test if conditions of t-test were not met
219 (normality studied using Shapiro-Wilk and assumption of homoscedasticity verified by Fisher-

220 Snedecor test). All enrolled participants were included in the analysis. However, a sensitivity analyses
221 provided similar results with completers only analysis.

222 **Results**

223 On the initially 100 recruited participants, 91 completed the first phase of the protocol (n=30Re;
224 n=28rE; n=33re) and 78 completed the entire study, phase 1 and 2 (n=24Re; n=24rE; n=30re). This
225 Figure 1 presents the flow-chart of the entire study. During phase 2, the mean compliance scores were
226 $54.6 \pm 22.1\%$ for Re, $52.7 \pm 26.1\%$ for rE, and $52.1 \pm 18.1\%$ for re, and did not differ between MetS
227 groups.

228

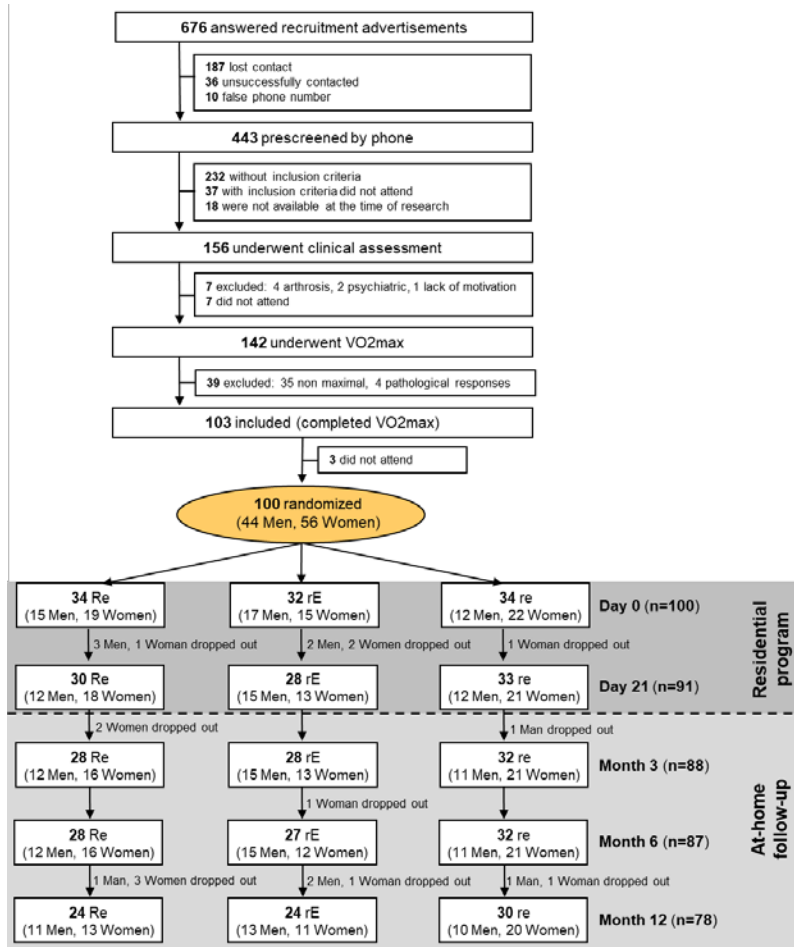


Figure 1. Flow-chart of the entire study. D0: Day 0; D21: Day 21; M3: Month 3; M6: Month 6; M12: Month 12;

Re: Resistance+endurance; rE: resistance+Endurance; re: resistance+endurance.

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Variations in body weight and composition throughout the protocol are presented in Table 1. As expected, there was a significant decrease in body weight, fat mass and fat-free mass during Phase 1 while physical activity and food intake were closely supervised ($p < 0.001$). From a quantitative standpoint, the mean fat mass loss during this period was about 3 kg, 3 kg and 2 kg in response to the

237 Re, rE and re conditions, respectively. Thus, its energy equivalent (9,300 kcal/kg) means that the
238 negative energy balance during Phase 1 largely exceeded the 500 kcal daily energy deficit that was
239 targeted at baseline.

240 Table 1 also shows that body fat loss continued in Phase 2 up to M3 ($p<0.001$). Specifically, the fat
241 loss of 2-3 kg that was achieved over the 70 days elapsing between the end of Phase 1 (D21) and
242 Month 3 was equivalent to a mean daily energy deficit of about 300 kcal/day. Beyond M3,
243 fluctuations of fat mass were small and no net noticeable additional fat loss was observed up to the
244 end of the protocol at M12.

245 As indicated, fat-free mass also decreased during the protocol (Table 1) ($p<0.001$). However, it is
246 noteworthy to emphasize that fat-free mass preservation was almost entirely achieved during the
247 whole protocol in the Re condition.

248

249

250 **Table 1.** Body weight and composition at different times during the protocol.

Variables	Time	Condition			Mixed Model		
		Re	rE	re	Group	Time	Interaction
Body weight (kg)	D0	85.4 ± 12.4	94.0 ± 13.7	89.0 ± 12.7		<0.001	
	D21	81.9 ± 11.7	87.1 ± 13.1	85.7 ± 12.1		D0 vs D21 ***	
	M3	79.1 ± 11.3	86.1 ± 12.3	82.6 ± 12.2	<0.05	D0 vs M3 ***	
	M6	80.4 ± 12.6	86.1 ± 13.6	82.8 ± 12.5	Re < rE *	D0 vs M6 ***	0.02
	M12	79.2 ± 11.9	84.9 ± 12.9	82.5 ± 12.7		D0 vs M12 ***	
						D21 vs M3 ***	
BMI (kg/m²)	D0	32.1 ± 3.9	34.4 ± 4.2	33.9 ± 4.0		<0.001	
	D21	30.8 ± 3.8	33.0 ± 3.9	32.7 ± 3.8		D0vs D21 ***	
	M3	29.6 ± 3.7	31.5 ± 3.6	31.6 ± 3.9	0.04	D0vs M3 ***	
	M6	30.2 ± 4.1	31.5 ± 4.0	31.7 ± 3.9	Re < rE*	D0vs M6 ***	0.02
	M12	29.9 ± 3.9	31.3 ± 4.0	31.8 ± 4.0	Re < re 0*	D0vs M12***	
						D21 vs M3 ***	
Fat mass (kg)	D0	27.7 ± 7.6	32.2 ± 7.7	32.3 ± 7.5		<0.001	
	D21	24.9 ± 7.1	29.3 ± 7.3	30.1 ± 7.3		D0vs D21 ***	
	M3	22.1 ± 6.9	26.3 ± 6.8	28.3 ± 6.8	<0.001	D0vs M3 ***	
	M6	23.1 ± 8.3	25.8 ± 7.6	28.0 ± 6.6	Re < rE **	D0vs M6 ***	0.03
	M12	22.7 ± 7.0	26.7 ± 8.1	28.5 ± 7.3	Re < re*** *	D0vs M12 ***	
						D21vs M3 ***	
FFM (kg)	D0	57.5 ± 10.8	61.8 ± 11.4	56.5 ± 10.7		<0.001	
	D21	56.9 ± 10.2	60.7 ± 10.8	55.6 ± 10.2		D0vs D21 ***	
	M3	56.5 ± 10.6	59.5 ± 10.9	54.5 ± 10.8	ns	D0vs M3***	<0.05
	M6	57.1 ± 10.4	60.3 ± 10.7	54.8 ± 10.6		D0vs M6 ***	
	M12	56.8 ± 11.1	58.1 ± 10.3	54.2 ± 10.8		D0vs M12 ***	
						D21vs M3 ***	
						D21vs M6***	
						D21vs M12**	
						M3vs M12 *	
						M6vs M12 **	

251 Values are means ± SD, D0, D21, M3, M6, and M12 refer to Day 0, Day 21, Month 3, Month 6, and Month 12, respectively; Re: Resistance+endurance; rE: resistance+Endurance; re: resistance+endurance. *p<0.05; **p<0.01; ***p<0.001.

254 Our analysis revealed a significant time effect (p<0.001) for daily energy intake with EI being

255 significantly higher at D0 compared with the other time points (D21 to M12) without any difference

256 between the other time points (D21 to M12). Although the analysis also shows a group effect with EI

257 being significantly higher in the re group compared with both Re and rE ($p<0.01$), there was no group
258 x tome interaction. Variations between time points were not significantly different between groups.

259 Figure 2 illustrates variations in plasma leptin during the protocol. As expected, there was a
260 considerable decrease in leptinemia during Phase 1 ($p<0.001$). This decrease continued between Day
261 21 and Month 3 ($p<0.001$). As for fat mass, there was no apparent clinically significant change in
262 leptinemia between M3 and M12. Variations in plasma ghrelin were also concordant with those of
263 energy balance up to M3 (Figure 2). Indeed, according to the literature cited above, the negative
264 energy balance that was imposed at the beginning of the protocol resulted in a significant increase in
265 plasma ghrelin at D21 and M3 compared to baseline values ($p<0.001$). The Leptin/ghrelin ration was
266 found significantly decreased at D21, M3, M6 and M12 compared with D0 in the rE and Re groups
267 (with no difference between D21, M3, M6 and M12) ($p<0.01$) but remained unchanged in the re
268 group.

269

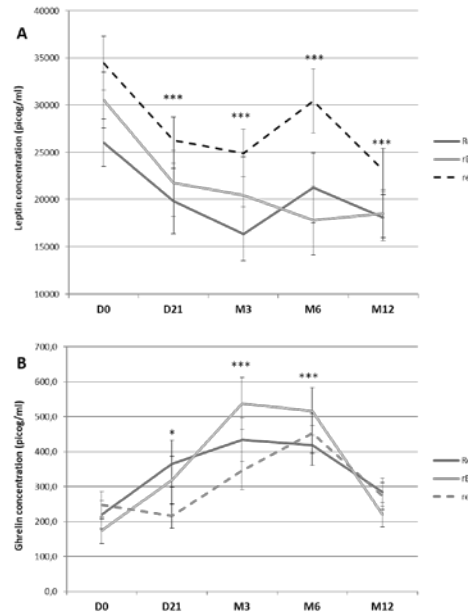


Figure 2. Plasma leptin (A) and ghrelin (B) concentrations before (D0) and after Phase 1 (D21) and after 3 (M3), 6 (M6) and 12 (M12) months (Phase 2) for the three treatment conditions: Resistance + endurance (Re), resistance + Endurance (rE), resistance + endurance (re). Values are means \pm SEM; *** $p < 0.001$ compared with D0; * $p < 0.05$ compared with D0.

However, contrary to other variables documented in this paper which reached a plateau at M3, there was a substantial decrease in ghrelinemia between M6 and M12 in each condition (Figure 1) to a level comparable to baseline values.

Discussion

The main objective of this study was to investigate the impact of different modalities of physical activity practice differing by the intensity of the exercise in combination with diet guidelines stimulus on body composition and some appetite-related hormones in overweight individuals. A particularity of the protocol was its implementation during 3 weeks of close in-house exercise and diet supervision

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284 that were followed by a second phase up to 12 months during which participants had to manage the
285 program individually. The beginning of the intervention in a controlled residential context promoted
286 a greater than initially expected energy deficit that was slightly more pronounced in response to high
287 intensity resistance or endurance exercise. After 3 weeks, once participants had the responsibility to
288 manage their exercise practice by themselves, daily energy balance and fat loss were reduced up to 3
289 months from which no further clinically significant morphological changes were observed up to the
290 end of the program. This apparent inability to further lose body fat after 3 months was accompanied
291 by substantial opposite changes in leptinemia which decreased, and ghrelinemia which increased in
292 response to fat loss. However, as further discussed in this section, a considerable decrease in plasma
293 ghrelin was observed between 6 and 12 months of follow-up when body weight and fat were relatively
294 stable.

295 Cross-sectional observations showed that vigorous physical activity is associated with reduced body
296 fatness, independently of the energy cost of activities (27). This has been corroborated by intervention
297 studies demonstrating that high intensity exercise accentuates body fat loss while increasing skeletal
298 muscle oxidative potential (3, 29). These observations are also concordant with results obtained in
299 standardized laboratory experiments indicating that calorie for calorie, high intensity exercise
300 influences global energy balance via postexercise adaptations in energy intake, appetite and resting
301 metabolic rate (14, 16, 32). From a clinical standpoint, these findings have contributed to the
302 dissemination of guidelines to exercise specialists focussing on the relevance to prescribe vigorous
303 physical activities as part of fitness programs. However, with respect to the management of excess
304 weight, these studies have not documented the issue as to "how much additional body fat loss" could
305 be achieved with high intensity physical activity in weight-reduced overweight individuals before the
306 occurrence of resistance to further lose body fat. In this regard, the methodology of the present study
307 contributed to answer this question by comparing the response of body fat over time in overweight

308 people subjected to different modalities of exercise practice. The results showed that when high
309 intensity exercise was included in the program, be it focussed on resistance or aerobic exercise, mean
310 body fat loss was accentuated by 1.5 to 2.0 kg before the achievement of a body weight plateau. This
311 reinforces the relevance to include vigorous physical activity in fitness programs provided that the
312 exercise stimulus is compatible with the health status of individuals. Our results moreover reinforce
313 the effect of high intensity exercise since our three groups responded similarly to the interventions in
314 terms of energy intake.

315 The findings outlined in the present study also reveal that irrespective to modalities of physical
316 activity practice, a body weight plateau is ultimately reached after some months of participation in a
317 program based on exercise and healthy eating, and as indicated above, this happened after 3 months
318 of intervention in our subjects. Interestingly, this was accompanied by a statistically significant and
319 quantitatively important decrease in plasma leptin, which is concordant with previously reported
320 variations in leptin (4). This is in agreement with many studies having demonstrated that a weight-
321 reducing program favors a decrease in plasma leptin, which is related to decreased thermogenesis (8,
322 28) as well as an increase in hunger sensations (17). This is also concordant with the demonstration
323 that leptin administration in weight-reduced obese individuals reverses these leptin-related changes
324 in thermogenesis and appetite (17).

325 The orexigenic hormone ghrelin has been shown to increase previously with weight loss in the
326 participants in other studies (7, 15, 19). This change represents a normal response which, together
327 with the decrease in plasma leptin, promotes body energy preservation in a context of energy
328 restriction. However, contrary to leptin which remained relatively stable when body weight had
329 stabilized after 3 months during the experimental protocol, a pronounced decrease in plasma ghrelin
330 was noted in each condition at the end of the study. Indeed, as depicted in Figure 2, plasma ghrelin

331 had then returned to values comparable to baseline levels when body weight and fat remained much
332 lower than their initial level. This unexpected finding may suggest that long-term physical activity
333 practice results in hormonal adaptations that facilitate over time the maintenance of reduced body
334 weight. Obviously, this hypothesis proposing that appetite control in the active person might be
335 facilitated on the long-term because a decrease in **ghrelin** deserves experimental confirmation. If
336 confirmed, this effect on **ghrelin** could provide a mechanistic explanation of the recognized benefit
337 of exercise to facilitate body weight/fat maintenance in weight-reduced obese individuals (10, 20,
338 26). Interestingly, our results also show a reduced Leptin/Ghrelin ration in response to the two
339 intensive interventions (Re and rE) but not in response to the re one. Although further research is
340 needed regarding the effect of exercise on this ratio, this is of importance since it might suggest that
341 intensive exercise might prevent patient for future weight regain compare to ow intensity
342 interventions. Indeed, as previously showed, a higher fasting L/G ratio has been found associated
343 with post-weight loss weight regain in overweight and patients with obesity (Crujeiras et al., 2014).

344 The present study has some strengths and limitations that are worthy of consideration. Among the
345 strengths, it is relevant to emphasize the duration of the protocol that was sufficiently long to permit
346 the occurrence of resistance to further lose body fat and to examine its related hormonal changes. The
347 high volume of training on Phase 1 composes an originality of the intervention made possible by the
348 residential nature of the program and the continuous presence of professionals. It remains however
349 hardly transferable in free-living condition as illustrated by the compliance results observed during
350 phase 2. This self-management of exercise guideline in Phase 2 might also represent a strength of this
351 study because of a better representativeness of what would happen under free-living conditions. With
352 respect to hormonal determinations, it is possible that, as described in the introduction of this paper,
353 the measurement of the active form of ghrelin would have contributed to a more thorough
354 documentation of the hormonal impact of our exercise intervention. However, it is unlikely that the

Commented [D7]: [Pre-treatment circulating leptin/ghrelin ratio as a non-invasive marker to identify patients likely to regain the lost weight after an energy restriction treatment.](#)
Crujeiras AB, Díaz-Lagares A, Abete I, Goyenechea E, Amil M, Martínez JA, Casanueva FF.
J Endocrinol Invest. 2014 Feb;37(2):119-26.

355 pronounced decrease in ghrelin that was found at the end of the study would not have been also seen
356 for acyl ghrelin. Another limitation is the use of self-reported dietary recall that might have led to
357 some underreported results which must be considered when interpreting the present results.

358 In summary, this study showed that increasing exercise intensity in an intervention combining
359 physical activity and diet guidelines promotes an accentuation of fat mass loss before body weight
360 reaches a new plateau in a reduced obese state. This occurrence of resistance exercise to lose fat was
361 associated with a decrease in plasma leptin and an increase in plasma ghrelin. Unexpectedly, ghrelin
362 almost returned to baseline values after several months of body weight stabilization. Further research
363 is needed to determine if this hormonal adaptation represents a long-term benefit of exercise
364 facilitating appetite control in active weight-reduced obese individuals.

365

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